Titin muscle protein levels may be non-invasive biomarker in DMD | Study: Urine test may be easier than bloodwork for young patients

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Levels of titin, a muscle protein, were found to be elevated in the urine of boys with Duchenne muscular dystrophy (DMD) — making it a potential, novel, non-invasive biomarker for the genetic disease, a study demonstrated.

Using a standard DMD mouse model, a lack of dystrophin, the protein missing in DMD patients, induced a rise in urinary titin, which was reversed by a treatment that rescued dystrophin production.

According to researchers, that means that titin levels may serve as a urinary biomarker in DMD — and, they noted, urine testing may be preferable to blood collection for younger Duchenne patients.

“To the best of our knowledge, this is the first report showing that elevated urine titin is directly caused by the lack of dystrophin in [DMD] mice, and urine titin responded to treatment,” the team wrote.

The study, “Urine titin as a novel biomarker for Duchenne muscular dystrophy,” was published in the journal *Neuromuscular Disorders*.

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The *DMD* gene encodes dystrophin, a protein that, together with other proteins, strengthens muscle fibers and protects them from wear and tear as muscles contract and relax. The most common type of muscular dystrophy, Duchenne is caused by variants in this gene that result in a dystrophin deficiency, marked by progressive muscle damage and wasting.

An elevated creatine kinase (CK) level in the bloodstream, a sign of muscle damage, is a widely used biomarker to diagnose DMD and monitor treatment efficacy. But because DMD is typically diagnosed in young patients, a less invasive urine test may be preferable to standard blood collection, the researchers noted.

Titin, also called connectin, is a very large protein that acts as a molecular spring responsible for the elastic nature of muscle fibers.

Recent studies suggest that urinary titin may be a useful biomarker in DMD because titin protein fragments have been found to be higher in the urine of DMD patients and mouse models.

To learn more, scientists at Takeda Pharmaceutical now collected and analyzed urine samples from 31 boys with DMD, ages 2-17, and 12 age-matched healthy controls. Urine titin concentrations were normalized to the urine creatinine levels (titin/cr), a marker for kidney function.

The mean urinary titin/cr concentration in DMD patients was 1992 picomole per mg (pmol/mg) — a level 326 times higher than the 6.1 pmol/mg mean concentration found in controls.

Urinary titin/cr levels decreased with age in patients but not in controls, and there were no differences in titin/cr regardless of walking abilities among DMD individuals older than 10.

The team tested the utility of urinary titin as an indicator of treatment efficacy using mdx mice, a widely used preclinical DMD mouse model. These mice carry a mutation in the *Dmd* gene (the mouse form of *DMD*), specifically in exon 23, a segment within the gene that carries the instructions to make dystrophin.

Like humans, urine titin/cr in mdx mice was significantly higher than in unaffected control mice at seven weeks, and remained high through 24 weeks (5.5 months). As expected, blood CK levels also were significantly elevated in the mdx mice.

The mice were then treated with an exon-skipping therapy — typically a treatment for human DMD patients — designed to mask mutations in exon 23, producing a smaller but functional version of the dystrophin protein.

Before treatment, mean urine titin/cr levels in the mdx mice were 9221.2 pmol/mg, dropping significantly to 113 pmol/mg after one week and 68.9 pmol/mg after two weeks. Blood CK results followed the same trend.

Treatment also stimulated the production of dystrophin to levels that were 51.5% of those found in control mice. Elevated urinary titin/cr correlated with lower dystrophin production.

> [Our work] suggests that elevated urine titin level might be a hallmark of DMD and a useful … marker for therapies designed to restore dystrophin levels.

The researchers noted that this study was the first to show that urine titin responded to treatment with an exon skipper.

“The present study suggests that lack of dystrophin directly induces elevations in urine titin in mdx mice and DMD patients,” the researchers wrote, adding that “urine titin might be a useful … marker for treatments that rescue dystrophin expression in skeletal muscle.”

Overall, the work “suggests that elevated urine titin level might be a hallmark of DMD and a useful … marker for therapies designed to restore dystrophin levels,”the team concluded.